The embryonic organization of the female genital disc and the origin and development of the female germline studied in mosaics of *Drosophila melanogaster*

Inaugural-Dissertation

zur Erlangung der philosophischen Doktorwürde
vorgelegt der
Philosophischen Fakultät II
der
Universität Zürich

von
Gertrud Schüpbach-Seiler
von Grosshöchstetten / BE

Begutachtet von Prof. Dr. R. Nöthiger, Zürich
und PD Dr. W. Janning, Münster (Westf.)

1979

Zürich 1978
W. Schneider, Druckerei, Zürich

The embryonic organization of the female genital disc and the origin and development of the female germline studied in mosaics of *Drosophila melanogaster*

Inaugural-Dissertation

zur Erlangung der philosophischen Doktorwürde
vorgelegt der
Philosophischen Fakultät II
der
Universität Zürich

von

Gertrud Schüpbach-Seiler

von Grosshöchstetten / BE

Begutachtet von Prof. Dr. R. Nöthiger, Zürich
und PD Dr. W. Janning, Münster (Westf.)

Zürich 1978

W. Schneider, Druckerei, Zürich

UNIV.
LEIDEN
BIBL.

ACKNOWLEDGEMENTS

I thank my teacher Prof. Rolf Nöthiger for his help and guidance during the course of this study. I am especially grateful to Dr. Eric Wieschaus for numerous suggestions and many fruitful discussions. I also thank Prof. E. Batschelet for statistical advice, as well as Drs. Ilan Deak, Andreas Dübendorfer, Kurt Dübendorfer, Emil Steiner and János Szabad for discussion of various results, Miss Annemarie Kohl for drawing the figures, Miss Margrit Eich for taking the photographs, and Miss Susi Schlegel for typing the manuscript.

Ich danke besonders auch meinen Eltern, die mir vor allem durch die liebevolle Betreuung meiner Tochter diese Arbeit ermöglichten.

This work was supported by grants 3.081.73 and 3.741.076 of the Swiss National Science Foundation to Prof. R. Nöthiger.

C O N T E N T S

I SUMMARY 1

II INTRODUCTION 3

III MATERIALS AND METHODS 6

IV RESULTS 8

1. Triplo-X lethality 8

2. Sterility of triplo-X females: The role of the genitalia and the germline 11

3. The embryonic origin of the female genital disc 13

3.1. Fate map 13

3.2. Number of precursor cells 14

3.3. The genital disc of normal gynandromorphs 15

4. The female germline 17

4.1. Origin and development 17

4.2. The sequence of stem cell divisions in the adult 18

V DISCUSSION 22

1. The triplo-X condition 22

2. The embryonic organization of the female genital disc 24

3. The female germline 28

3.1. Number of precursor cells 28

3.2. Cell lineage relationships 33

3.3. Stem cell divisions in the adult 34

REFERENCES 36

TABLES

FIGURES

SUMMARY

The female genital disc of Drosophila melanogaster gives rise to the genitalia as well as the analia of the adult. This study describes the embryonic organization of the female genital disc. It also reports on the embryonic origin of the female germline and on the dynamics of germ cell production in the adult female. All results were obtained indirectly using the information on cell lineage relationships provided by genetic mosaics.

Mosaic females were produced either by the early loss of a ring-X chromosome ($\hat{X}\hat{X}$/R(X)-$\hat{X}\hat{X}$/0 = triplo-X-diplo-X mosaics) or by the early loss of a marked Y-chromosome ($\hat{X}\hat{X}$/Y-$\hat{X}\hat{X}$/0 mosaics). The mutations chosen as markers of the chromosomes allowed us to analyse the genotype of various internal and external structures in these mosaics. The mosaic patterns were recorded and an embryonic fate map was constructed (Fig. 1). In this fate map the precursors of the germline were located on the line of right/left symmetry of the embryo, posterior to all other adult structures. The precursor cells of the genital disc were shown to derive from a region of the embryo also situated posteriorly on the embryo's axis of symmetry, but more ventral and anterior to the precursors of the germline. The size of the embryonic precursor areas of the germline and of the genital disc was estimated. Since the germ cell population was mosaic in 22%-29% of the mosaic females (Table 2) the precursor area of these germ cells was estimated to contain 6-16 cells at the blastoderm stage. Judged from its high frequency of mosaicism (34.5%) the precursor area of the female genital disc was estimated to contain 15-20 cells at the blastoderm stage.

Within the ovaries of females with mosaicism in their germline no frequently repeated pattern could be found which would have indicated any cell lineage restriction among the germ cells of the early

embryo. Single ovarioles often contained oocyte-nurse cell cysts of different genotypes (Fig. 5) which shows that the stem cells in the germaria of these ovarioles were of polyclonal origin. An analysis of the sequential pattern of the oocyte-nurse cell cysts in such ovarioles revealed that neighbouring cysts tend to be of the same genotype. This suggests that the stem cells of the adult ovary preferentially divide in bursts, one of them giving rise to two, three, and sometimes even more cystocytes in a row.

Within mosaic genital discs the only pattern that was exactly repeated several times was the complete separation of the genitalia (excluding the parovaria) from the analia. In these cases the parovaria were of the same genotype as the analia, indicating a closer embryonic relationship between the parovaria and the analia than between the parovaria and the genitalia. From a comparison with the mosaic patterns found in normal gynandromorphic flies it was concluded that the precursor cells of male genital structures lie between the precursors of female genitalia and analia (Table 8, Fig. 4). These precursor cells of male genital structures do not appear to give rise to scoreable adult structures in a female animal which would explain why in mosaic females cases of complete separation between female genitalia and analia should occur more often than any other mosaic pattern.

In addition, the triplo-X-diplo-X mosaic females yielded some information on the cause of the lethality and the sterility which are observed in flies completely composed of triplo-X cells (metafemales). There exists a lethal focus which lies close to the ventral thoracic discs in the early embryo and causes the death of an animal when it is of triplo-X genotype. The focus of sterility resides in the genitalia, since flies with triplo-X genitalia are incapable of laying any eggs, regardless of the genotype of their ovaries.

II INTRODUCTION

In Drosophila three different cell types take part in the formation of the reproductive system: the cells of the germline, the somatic cells of the gonads, and the cells of the genital disc which form the internal and external genitalia and analia of the fly. The development of these three cell types has been described in a number of morphological and histological studies. Already at the blastoderm stage a morphologically distinct group of cells called pole cells are formed at the posterior pole of the embryo (Sonnenblick, 1950; Turner and Mahowald, 1976; Zalokar and Erk, 1976). In various experiments it has been shown that this group of cells contains the precursors of the adult germline (Huettner, 1923; Geigy, 1931; Poulson, 1947; Poulson and Waterhouse, 1960; Fielding, 1967; Illmensee, 1973). During midgut invagination the pole cells move inside the embryo and after penetrating the wall of the gut some of the cells reach the body cavity of the embryo where they are surrounded by the mesodermally derived somatic cells (Sonnenblick, 1941). Together they form the embryonic gonad which grows by cell division during larval development and differentiates during metamorphosis into the adult gonad. In the female fly, the germ cells will produce the cystocytes which will develop into the oocyte-nurse cell cysts observed in the ovarioles of the ovary and finally differentiate into the mature egg cell. The somatic cells will form the gonadal sheath and in addition surround each cystocyte with a single layer of follicle cells (King, 1970; Mahowald, 1972).

The genital disc of both sexes can already be observed at the end of embryogenesis to be attached to the ventral side of the larva, close to the anus (Laugé, 1968). This disc contains the cells which will form the genital duct and accessory glands as well as the anal plates and the endgut (Dobzhansky, 1930; Hadorn and Gloor, 1946; Ursprung, 1957; 1959).

It was the purpose of this work to obtain more information about the way the precursor cells of the female genital disc and the female germline are organized in the early embryo. In particular we wanted (1) to gain an estimate of the size of the areas in which these precursor cells are located, (2) to place these areas on an embryonic fate map with respect to the precursor areas of other adult structures, and (3) to study the cell lineage relationships between the cells lying within these embryonic precursor areas.

Different studies using mosaic flies generated by the early loss of a chromosome have already led to general locations of the precursor cells for both the genital disc and the germline on the embryonic fate map (Garcia-Bellido and Merriam 1969; Hotta and Benzer, 1973; Janning, 1974; Gehring et al., 1976; Nissani, 1977a). More detailed analyses were, however, prevented by the fact that the mosaic flies studied consisted in part of male and in part of female cells and often showed neither a complete male nor a complete female reproductive system but a rather complex mixture of both. To circumvent this problem a genetic system had to be devised in which the loss of a chromosome did not change the sex nor interfere with the normal function of the cells, but merely resulted in a phenotypic marking of the different cells with neutral cell markers.

In this study two types of mosaic females were produced (see Materials and Methods). A detailed embryonic fate map of the female genital disc was constructed and the size of the total precursor area was estimated. The fate map was compared to the fate map of the genital disc derived from normal gynandromorphs. This provided further information on the origin of the sexual dimorphism observed in the genital disc derivatives of the adult Drosophila (Nöthiger et al., 1977).

With respect to the germline it was possible to locate the embryonic precursor area and to estimate its size in the early embryo. In contrast to most other tissues of the adult fly the germ cells continue

to divide and yield genetically analysable products (oocytes or offspring) throughout the entire life of the fly. By determining the sequence of oocytes with different genotypes in mosaic ovarioles this study could therefore be extended to analyze aspects of the stem cell divisions in the adult ovary.

One type of mosaics used in this study were triplo-X-diplo-X mosaic females generated by the early loss of an unstable ring-X chromosome in triplo-X metafemale embryos. Completely triplo-X metafemale flies are barely viable and always sterile (Beadle and Ephrussi, 1937). Since our mosaics provided us with all the different combinations of triplo-X and diplo-X tissue we were also able to analyze the causes of the lethality and sterility of triplo-X metafemales.

III MATERIALS AND METHODS

Mosaic females were obtained by early loss of a chromosome in two different crosses:

1) The cross C(1)RM, y v f mal/Y x In(1) w^{vC}/Y.y^{+} yielded triplo-X-diplo-X mosaic females (for a description of all mutations see Lindsley and Grell, 1968; In(1) w^{vC} also Hinton, 1955; Hall et al., 1976). These mosaics ($\hat{XX}$/In(1)-$\hat{XX}$/O) were individually mated to y v f mal/Y males. This allowed us to classify the progeny of each female as to whether it arose from an originally triplo-X or a diplo-X germ cell: progeny deriving from triplo-X germ cells either carried the ring-X chromosome or had phenotypically v mal^{+} eyes, due to the maternal effect of the mal^{+} gene on eye colour, which is germline dependent (Marsh and Wieschaus, 1977).

2) For the second type of mosaics $\hat{XX}$, y v f mal/Y females were crossed to males y/y^{+}Y.mal^{+}; pal/pal. The mutation pal (paternal loss; Baker, 1975) causes loss of the Y-chromosome in $\hat{XX}$/Y zygotes with low frequency. In our cross this loss resulted in females which were mosaic for the genetic markers y and mal. Those females were singly mated to y/Y males and the progeny classified as to whether they arose from $\hat{XX}$/y^{+}Y.mal^{+} oogonia or from $\hat{XX}$/O oogonia. For this classification we used the presence or absence of the marked Y-chromosome among the male progeny and the maternal effect of the mal^{+}-gene among the female progeny.

The flies were raised on standard Drosophila medium containing cornmeal, sugar, agar, yeast, and Nipagin, and were kept at 25°C, or at room-temperature in the case of the crosses yielding the triplo-X-diplo-X mosaic females and the normal gynandromorphs.

All mosaic females were mated between the 2nd and 5th day of their adult life. The daily egg production was then protocolled for at least ten days. From half the tested females a fraction of the eggs was put on moist green blotter paper, and hatched larvae as well as unhatched eggs were counted individually for each female and day. When such unhatched eggs turned brown, they were further classified as embryos that had at least reached the cellular blastoderm stage (Fritz-Niggli, 1955). After this progeny test each female was dissected and her genitalia and ovaries were histochemically stained for aldehydeoxidase (AO) activity, following the procedure of Janning (1976). Since cells homozygous for mal lack AO activity (Janning, 1972) this gene was used as a cellmarker for internal tissues. With this technique it was possible to clearly distinguish the genotype of the oocytes and nurse cells in the vitellaria (Fig. 5) of the adult ovaries up to stage 9-10 (staging according to King, 1970), as well as the genotype of most cells in the genitalia. Some small regions in the vagina could not be analyzed because they were AO-negative even when wild-type. This also applies to the germ cells in the germaria and all mesodermal cells in the ovary whose genotypes therefore could not be revealed by staining.

The cuticular parts were cooked in 10% NaOH and mounted in Faure's solution for inspection under a compound microscope.

In order to compare certain results obtained with mosaic females to normal gynandromorphs, the cross y v f mal/y v f mal x In(1)w^{vC}/Y.y^{+} was carried out. From the gynanders collected in this cross only the cuticular parts were analysed.

IV RESULTS

1. Triplo-X lethality

Metafemales which consist of triplo-X tissue (3 X-chromosomes, 2 sets of autosomes) very rarely survive through metamorphosis (Brehme, 1937). Accordingly, the cross $\hat{XX}$, y v f mal/Y x In(1) w^{vC}/Y.y^{+} yielded only few adult metafemales (1%). The bulk of the offspring consisted of $\hat{XX}$/Y.y^{+} females (95%) and, apart from rare $\hat{XX}$/0 females (< 1%), of 480 triplo-X-diplo-X mosaic females representing 4% of the total adult progeny of this cross. This yield of mosaics was significantly lower than that of normal gynandromorphs from the control cross y v f mal/y v f mal x In(1) w^{vC}/Y.y^{+}. Here, the gynandromorphs amounted to 10.1% of the X/Y.y^{+} progeny, which is the class of offspring corresponding to the $\hat{XX}$/Y.y^{+} females of the first cross. The triplo-X constitution, which causes lethality in non-mosaic animals must therefore also cause some lethality in triplo-X-diplo-X mosaic flies. To see whether this lethality arises only when particular regions of the fly are of triplo-X genotype, the frequencies were determined with which different adult structures were completely composed of triplo-X cells. This was done in 260 of the triplo-X-diplo-X mosaics (Table 1). The lowest frequency was found for the legs, in particular the forelegs, whereas structures more anterior, more posterior or more dorsal were more often of triplo-X genotype. These results point to the existence of a lethal focus in the ventral thoracic region of an embryo which causes the death of an animal, when it is of triplo-X genotype. The flies with a triplo-X foreleg can be interpreted as those cases where the mosaic border separated the foreleg completely from the lethal focus. The lethal focus can then be visualized as an area extending across the ventral midline connecting two points which lie

about 2 sturts ventral of the edge of the forelegs (footnote[1]). It can also be visualized as two unconnected areas, one on each side of the embryo. In both cases one has to assume that the triplo-X lethal focus is domineering (Hotta and Benzer, 1973), such that one side of the focus being triplo-X (or one of the foci in the case of the two unconnected foci) leads to the death of the animal. This conclusion stems from the fact that completely triplo-X legs are very rare and no fly with two triplo-X forelegs or second legs was ever observed in this study.

It became important to consider the influence of this lethal focus on frequencies of mosaicism or sturt distances measured in triplo-X-diplo-X mosaic flies. For reasons of simplicity we shall speak here of "two foci" rather than of "the two sides of the one focus". If both foci are of the same genotype, the cases where both foci are diplo-X (survival of the embryo) should equal the cases where both foci are triplo-X (death of the embryo). These cases should therefore not alter the frequencies with which structures will be found mosaic among the surviving flies. However, all of the embryos in which the mosaic border separates the two foci will die. Any area which maps close enough to the lethal foci to be frequently mosaic when the mosaic border runs between the foci should therefore be less

---

footnote[1]: In the case of the triplo-X-diplo-X mosaic females it is impossible to perform the correct calculation for lethal focussing used in studies by Hotta and Benzer (1972) or Bryant and Zornetzer (1973) since their calculation is based on a comparison between two types of X/R(X) - X/O mosaics which either do or do not carry a lethal mutation in their hemizygous tissues. In our study $\hat{X}\hat{X}$/R(X) - $\hat{X}\hat{X}$/O mosaics can only be compared to X/R(X) - X/O mosaics with which they have no cells with exactly the same genetic constitution in common.

frequently mosaic among the surviving flies than an equally large area which maps far apart from the lethal foci. A difference in sturt distances between normal gyandromorphs and triplo-X-diplo-X mosaics was indeed found for the distances measured between the right and left legs (right-left distances of 1^{st}, 2^{nd}, and 3^{rd} leg in normal gynandromorphs: 31.3; 36.1; 37.9; in triplo-X-diplo-X mosaics: 21.9; 25.5; 31.0). Apart from these, no other distances or frequencies of mosaicism were significantly different in the triplo-X-diplo-X mosaics compared to normal gynandromorphs (Table 2). This indicates that the lethal foci occupy a small area on the embryonic fate map. The good correspondence between the mosaic frequencies of adult cuticular structures measured in triplo-X-diplo-X mosaics and in normal gynandromorphs made it unnecessary to introduce any correction factors when the results of the two mosaic sxstems were further compared.

In addition to analysing the lethality of whole animals the influence of the triplo-X constitution on the developmental "fitness" (= cell survival and growth rate) of cells within imaginal discs could also be studied in the triplo-X-diplo-X mosaics. In the adult flies most triplo-X wings and in some cases also the rare triplo-X legs were abnormally formed. In mosaic discs the triplo-X cells seemed, however, not to exhibit a strong reduction in fitness. In mosaic first or 2^{nd} legs on an average 42% of the bristles were of triplo-X genotype. In mosaic 2^{nd} and 3^{rd} tergites this number amounted to 49%, and in mosaic genital discs 43% of the bristles were of triplo-X genotype. These values only allow for a very minor developmental disadvantage of triplo-X cells during the whole development of the corresponding imaginal discs or histoblasts.

2. Sterility of triplo-X females: The role of the genitalia and the germline

The rare surviving triplo-X metafemales were invariably sterile. Of 40 metafemales none laid any eggs, and most of them died within 5 days of adult life. Of the tested triplo-X-diplo-X mosaic females 129 out of 205 gave rise to progeny, the rest remained sterile within the test period of 10-12 days. The egg production of each female was compared to the genotype of her ovaries and genitalia (for a description of the female genitalia see Fig. 2a). Flies with completely triplo-X genitalia never laid any eggs, regardless of the genotype of their ovaries (Table 3). Also, in such flies we never observed any egg having entered the oviduct, but instead found a large number of mature eggs accumulated in the ovaries. In contrast, mosaic flies with purely diplo-X genitalia laid the same number of eggs as non-mosaic controls (Table 3). One reason for the sterility of triplo-X females must therefore be ascribed to their triplo-X genitalia.

Many flies (40 out of 48) with mosaic genitalia did lay eggs (Table 3). The rate of egg production could not be correlated with the genotype of any particular structure (Fig. 2a) within the genitalia. Each genital structure was triplo-X in 7-10% of the flies which laid eggs, or diplo-X in 7-10% of the flies which laid no eggs. Rather, the egg production in females with mosaic genitalia depended on the total amount of triplo-X tissue in the genitalia. Flies with less than 20% triplo-X tissue anywhere in the genitalia laid a normal amount of eggs, whereas flies with more than 80% triplo-X tissue were incapable of laying more than one or two eggs. The reduced egg production in females with mosaic or triplo-X genitalia was not due to an incapacity to mate. When the receptacula of 30 tested flies which had laid only few or no eggs were examined, motile sperm was detected in 24 of them, including 16 whose genitalia were completely triplo-X.

As long as a female had diplo-X genitalia, her rate of egg production was relatively unaffected by the genotype of the germ cells, although females with triplo-X germ cells did lay somewhat fewer eggs than females with a diplo-X germline (Table 4). The eggs derived from triplo-X germ cells, however, did substantially less well during later development, and of 100 such eggs on an average only 2 adult offspring survived. This represents a tenfold decrease when compared to the 24 adult offspring obtained from eggs laid by mosaic flies with diplo-X germ cells. Among the zygotes produced by all different tested females a certain amount of lethality is expected due to their chromosomal constitution (triplo-X and In(1) w^{vC}/Y from triplo-X germ cells, or triplo-X and Y/O or Y/Y from diplo-X germ cells). Among the progeny of females with triplo-X germ cells a further reduction in viability would be expected, due to a dominant lethality of the ring-X chromosome (Hinton, 1955) which was estimated in a control cross to kill 54% of X/In(1) w^{vC}-zygotes. Altogether, this would still not account for the developmental failure of 97% of the eggs formed by triplo-X germ cells. The main difference in survival, as compared to eggs from diplo-X germ cells, resulted from lethality in early development. In all cases about one third of the first instar larvae developed to adults (Table 4, e and f).

3. The embryonic origin of the female genital disc

3.1. Fate map

The genital disc gives rise to cuticular (external) as well as to "soft" (internal) structures. The mutations chosen as markers for the mosaic females allowed us to study the embryonic origin of both external and internal tissue. In 309 triplo-X-diplo-X mosaic females and in 54 pal-induced mosaic females the genotype of all derivatives of the genital disc as well as the genotype of several other abdominal structures was examined. A fate map of the female abdomen was constructed using the data of the triplo-X-diplo-X mosaics according to standard procedures (Hotta and Benzer, 1973). The precursor cells of the female genital disc mapped to a region about 27 sturts apart from the precursors of the 7^{th} sternites, close to the ventral midline. An approximately equal ventral and posterior position has been assigned to the precursor cells of the genital disc of gynandromorphs (Hotta and Benzer, 1973; Janning, 1974; Gehring et al., 1976) and of male flies (Gehring et al., 1976; Nissani, 1977a), and has also been confirmed when the data of the other types of mosaics produced in this work were analyzed.

Within the genital disc derivatives of the mosaic females several external and internal landmarks were chosen (Fig. 2a) and an intra disc fate map was constructed (Table 5, 6; Fig. 2b). Since the values measured in the 54 pal-induced mosaics never differed significantly ($p \leq 0.05$) from those measured in the 309 triplo-X-diplo-X mosaics (Table 5 and 6) the text will only refer to the data of the triplo-X-diplo-X mosaics. The landmarks of the genitalia and those of the analia fell into two distinct groups. Within each of these groups the sturt distances ranged from 0.1 sturts to 9.0 sturts in the genitalia (with the exception of the parovaria), or to a maximum of 6.7 sturts within the analia. Distances involving structures from both groups, however, were all larger than 9.4 sturts. Of all internal genital structures only the parovaria mapped closer

to the analia than to the genitalia (Table 5), although in an adult female they are connected to the middle part of the genital duct and are completely separated from the anal plates (Fig. 2a). The close relationship between parovaria and anal plates is also demonstrated in those 9 females in which the mosaic border ran between the genitalia and the anal plates. In 8 of these the parovaria were of the same genotype as the anal plates, while in one case they were mosaic, but never were they of the same genotype as the genitalia and different from the analia.

Within the genitalia or analia there were no frequently outlined genotypic borders. In particular there was no clear separation between external and internal structures. Right-left distances were also very often extremely small, as for example between the right and left landmarks of the oviduct (D_1-D_1, D_2-D_2, Table 5). This was, however, not a general characteristic of internal structures, e.g. the landmarks on the right and left side of the lower half of the genital duct (= vagina, V_1-V_1, V_2-V_2, Table 5) were in 10% of the cases of different genotype.

3.2. Number of precursor cells

In gynandromorphs the frequency with which a structure is mosaic depends on the size of the area from which its precursor cells are drawn (Hotta and Benzer, 1973). However, frequencies of mosaicism cannot be directly converted into numbers of precursor cells since they are relative values and not linked to a specific time and therefore to a specific cell number in development. The simplest way of evaluating the size of such a precursor area is to compare the degree of mosaicism of the corresponding adult structure with that of other structures. The relative size scale one obtains between the different structures applies for the whole time during which the precursor cells (or nuclei in an earlier stage) develop at the same rate. For precursors of imaginal discs and abdominal histoblasts this should be the case at least up to the blastoderm stage and probably also during the next few hours of embryonic development (Wieschaus, 1978).

The precursors for the entire female genital disc including both external and internal derivatives seem therefore to be drawn from an area bigger than the foreleg (Table 2). The area for the entire external and internal female genitalia falls into the size class of one single thoracic disc, whereas the genitalia or analia on one side of the fly arise from a somewhat smaller area. The area from which both external and internal structures are derived always exceeds the area giving rise to external structures only. Since the precursor area of a single foreleg has been estimated to include around 10 cells at the blastoderm stage (Wieschaus and Gehring, 1976 a, b) one can now estimate the total number of blastoderm cells within the precursor area of the genital disc to be around 15 (Table 7). The sizes of some other precursor areas within the genital disc were estimated in the same way and the results are also given in Table 7.

3.3. The genital disc of normal gynandromorphs

The analysis of the derivatives of the genital disc in normal gynandromorphs should yield a fate map of the total genital-anal area in the embryo.

Of the 512 normal gynandromorphs 138 (= 26.9%) showed mosaicism in their external genital disc derivatives. This value is not different from the values found in the external genital disc derivatives of the female mosaics (triplo-X-diplo-X mosaics 28.2%, *pal*-induced mosaics 31.5%, Table 2).

When the mosaic patterns in these gynandromorphs were recorded there arose difficulties which had not existed when the patterns in the female mosaics had been scored. Sometimes it was ambiguous to decide whether only one or both sides of a structure were mosaic, expecially when structures were split into several separate pieces, as for instance in the 7 flies with more than two pieces of lateral plate, each associated with a piece of clasper (for a description of the derivatives of the male genital disc see Fig. 3). In addition, 25 of these 138 mosaics displayed structures that were abnormally enlarged, mainly claspers or vaginal plates which carried far more bristles than normal. These enlarged structures sometimes gave the impression

of small duplications. Eleven of these flies showed a duplication of the ventralmost part of the clasper. These abnormalities were always scored as mosaicism of the corresponding total structure (Table 8a). The genotype of the right and left side was decided mainly by orienting the genitalia according to the axis set by the right and left halves of the anal plates, in which normally no such supernumerary strucutres were observed.

A fate map of the genital and anal structures was then constructed (Table 8, Fig. 4b). The female vaginal plates and the 8^{th} tergites mapped farthest away from the anal plates, the penis closest to the anal plates, whereas the hypandrium and the male genital plate (Fig. 3) took an intermediate position.

4. The female germline

With respect to the germline the results of triplo-X-diplo-X mosaics and *pal*-induced mosaics did in most cases not differ from one another. Hence, they have been pooled in this section unless otherwise indicated.

4.1. Origin and development

The genotype of the germ cell population in the ovaries could be determined either by histochemical staining of the oocyte-nurse cell cysts in the ovary or by phenotypic analysis of the resulting progeny (Table 2).

Both evaluations revealed females with mosaicism in their germline. The difference in frequencies obtained by the two methods is due to the fact that the offspring sometimes failed to demonstrate mosaicism in the germline of the female, although the histochemically stained ovaries contained oocyte-nurse cell cysts of both genotypes. The results of the histochemical staining are more representative of the actual stem cell population, since they are based on examination of some 200 oocytes per females, whereas the average numbers of adult offspring only ranged from 2.9 to 40 (Tab. 4, f). In addition, any meiotic and post-meiotic selection of particular genotypes makes an estimation of the stem cell population based on adult offspring less accurate.

In the fate map of the abdomen the germ cell population was assigned the posterior-most position (Fig. 1) which confirmed earlier reports (Janning, 1974; Gehring *et al.*, 1976; Nissani, 1977a). Within mosaic ovaries the fraction of cells with $\hat{X}X/0$ genotype varied from 3%-97% with a rather even distribution over the whole range (Fig. 6). Among the 79 females with mosaic gonads there was none with a strict right/left separation between the germ cells of the two ovaries. 59 were mosaic in both ovaries and 20 were mosaic in only one gonad. The last two statements imply a common cell lineage for the right and left germline in the embryo up to the blastoderm stage, which was to

be expected, given the observed position of the pole cells as a single cluster at the posterior end of the embryo.

Each ovary of an adult female consists of some 17 ovarioles. In the vitellarium of each ovariole the genotype of about six consecutive oocyte nurse cell cysts could be clearly determined by histochemical staining (Fig. 5; Table 9). With one exception all 138 mosaic ovaries contained at least one ovariole which showed cysts of both genotypes (Fig. 5). These mosaic ovarioles must have incorporated at least two cells of different genotype into their germarium at the time of their formation.

The ratio of cysts with different genotypes in the mosaic ovarioles gives the relative rate of division of the corresponding stem cells. In the triplo-X-diplo-X mosaics there were 1224 diplo-X and 1050 triplo-X cysts in the mosaic ovarioles. This suggests that the cell cycle of triplo-X germ cells in the adult ovary is longer by a factor of 1.16 than that of diplo-X germ cells. This small difference will not appreciably affect the pattern of cyst production within an ovariole (see p. 19). Yet, if triplo-X germ cells already had the same relative division rate during the preadult development of the gonad, i.e. during the phase of logarithmic growth (Wieschaus and Szabad, in press) this difference would amount to a considerable predominance of diplo-X germ cells in the mature ovary. Starting from a 1:1 ratio at blastoderm, 4 cell divisions between that stage and pupal gonad (Wieschaus and Szabad, in press) will lead to a 0.55:1 ratio of triplo-X to diplo-X germ cells at the time when ovarioles are formed. This agrees reasonably well with our observation that the mosaic ovaries in total contained 0.60:1 triplo-X cysts (3318) to diplo-X cysts (5502).

4.2. The sequence of stem cell divisions in the adult

The oocyte-nurse cell cysts in an ovariole reveal not only the genotype of the "invisible" stem cells in the germarium and their relative

rate of division. By their linear arrangement they also provide a record of the sequence with which the different stem cells in a given ovariole divide. The number of stem cells per ovariole has been estimated to be very small (King, 1970; Carpenter, 1975; Wieschaus and Szabad, in press). None of 455 mosaic ovarioles contained $\underline{mal}^+$ and $\underline{mal}^-$ cysts arranged in a strictly alternating fashion, such as we would expect assuming two stem cells in the germarium which divided alternately. A further analysis of the sequential patterns in mosaic ovarioles (Fig. 5) showed that only 32.7% of these could be explained by assuming regularly alternating divisions of three to five stem cells per ovariole. One therefore has to conclude, that the cell cycle of the stem cells is more irregular, and that individual stem cells in the same ovariole do not divide in strict alternate sequence.

It was tested whether the stem cells divide randomly, which would mean that after a particular stem cell z has divided, all stem cells in the germarium (including z) have again an equal chance of producing the next cystocyte. To answer this question the frequency with which neighbouring cysts in mosaic ovarioles were of the same genotype was determined. The observed frequency of 71% of identical neighbours (1612 out of 2275 neighbouring cysts had the same genotype) significantly surpasses the expected frequency of 50%-57% (see footnote[2]).

---

footnote[2]: random division

Assuming all germaria to contain 2 stem cells, in a mosaic ovariole they would be of different genotype and the probability of producing two adjacent cysts of the same genotype is $2 \cdot (1/2)^2 = 50\%$. With three stem cells per ovariole one would be of different genotype than the other two in a mosaic ovariole, and the probability for two adjacent cysts to be of the same genotype is $(1/3)^2 + (2/3)^2 = 55.6\%$. The maximum of this function is reached with 5 stem cells per ovariole (57.3%); larger numbers tend again towards 50%.

This departure from randomness means that a stem cell often divides more than once before another stem cell in the same germarium gives rise to a cyst, since this is the only way to produce more than 57% neighbouring cysts of the same genotype. Comparing the genotypes of cyst 1 and 3, 2 and 4 etc. we find 1131 pairs of cysts out of 1820 (62%) to be of the same genotype. When we proceed to compare cyst 1 with 4, 2 with 5 etc., they show the same genotype in 48% of the cases. Going further, the first and fifth cyst are of the same genotype in 42%, the first and sixth in 32% of the cases. Stem cells, therefore, preferentially divide in bursts, giving rise to two, sometimes three or four cysts at a time. But after having gone through such a burst, the fifth and sixth cysts are then usually produced by the other stem cells within the same ovariole. This pattern is not rigid, and stem cells sometimes give rise to only one cyst at a time. In 142 out of 1820 cases (8%) a cyst of one genotype was flanked by cysts of the other genotype (Fig. 5c). Such a pattern cannot be produced unless a stem cell divides just once. Not all such single divisions, however, necessarily lead to this particular pattern (see footnote[3]).

---

footnote[3]: switches of division between stem cells

Assuming two stem cells per germarium in a mosaic ovariole, every alternation in division between the two cells would result in a change in cyst genotype. In this case a cell which divides only once would always produce the pattern of a single cyst flanked by cysts of opposite genotype. With three stem cells per germarium we expect that the dividing cell is of the minority genotype in a third of the cases. Then a switch to another stem cell will always result in a change in genotype of the following cyst. Likewise, if this cell divides only once, we would invariably obtain the pattern of a "single" cyst. In two thirds of the ovarioles, however, there would be another stem cell with the same genotype as the one just dividing, and then only half the switches would become detectable. We would observe a single division of this cell only if immediately before and after this single division the cell of the minority genotype had divided. The probability

Finally, we wanted to know how many stem cells are involved in producing a given number of eggs. Assuming 2-5 stem cells per ovariole, we have calculated (see footnote[3]) that between 30 and 48 stem cells are involved in the production of 100 oocytes. Between 8 and 17 stem cells divide just once, whereas the rest gives rise to two, three or even four cysts in a row.

---

footnote[3] (contin.):
of this is $(1/2)^2$. In total: 2/3 of all switches would be detected ($1/3 + (2/3 \cdot 1/2)$ and only 1/2 of all single divisions would produce a "single" cyst flanked by neighbours of different genotype ($1/3 + (1/2 \cdot 2/3 \cdot 1/2)$). For larger numbers of stem cells per germarium the probabilities of observing a change in genotype or a "single" cyst tend towards minima of 1/2 for a change and $(1/2)^2$ for a "single" cyst.

V DISCUSSION

1. The triplo-X condition

In this analysis three distinct effects of the triplo-X condition have been found:

1) In the thoracic region of the embryo there exists a domineering lethal focus which must be mainly responsible for the lethality observed in triplo-X metafemales (Brehme, 1937).

2) Eggs will not enter the female oviduct if it consists of more than 80% triplo-X tissue. This is the main reason for the sterility of the rare surviving metafemales (Beadle and Ephrussi, 1937).

3) Eggs derived from triplo-X germ cells can hardly support normal development, most of them never complete embryogenesis, many never even develop to the cellular blastoderm stage. This represents a second reason for a triplo-X sterility, or, at least a drastical reduction in fertility, which is, however, only expressed if the genitalia of the female are diplo-X, such that eggs can be deposited.

Apart from these strong defects the triplo-X condition had some less definable effects on the formation of wings and legs, and led to a somewhat reduced rate of cell division in the female germline.

The cause off all these effects must lie in the abnormal chromosomal constitution with 3 X-chromosomes and two sets of autosomes. In Drosophila dosage compensation has been shown to occur such that flies with two X-chromosomes will exhibit the same amount of X-linked gene products as flies with only one X-chromosome (Muller et al., 1931; Seecof et al., 1969; Faizullin and Gvozdev, 1973; Lucchesi, 1977). From the literature it is not clear yet whether and to what extent dosage compensation occurs in metafemales. Some authors describe that

the level of X-linked enzyme activity in triplo-X females is the same as in normal diplo-X females (Lucchesi et al., 1974; Stewart and Merriam, 1975), others found that metafemales exhibit at least in some X-linked enzymes a higher level of activity than do diplo-X females (Faizullin and Gvozdev, 1973; Ananiev et al., 1974). In the triplo-X-diplo-X mosaics examined in this study most of the cells and tissues seemed to survive and develop normally when they were of triplo-X genotype. This would argue against a major imbalance between X-linked and autosomal gene products and rather indicate that some type of dosage compensation can occur in triplo-X tissues. However, the fact that the above mentioned specific effects are observed in metafemales and in certain triplo-X-diplo-X mosaics can only be explained by assuming that dosage compensation does not work as perfectly in triplo-X tissues as it does between normal males and females, and that some cells or tissues are more sensitive to this imbalance than others.

2. The embryonic organization of the female genital disc

From the time when segmentation first becomes apparent in a Drosophila embryo up to metamorphosis the abdomen consists of eight visible segments (Poulson, 1950; Turner and Mahowald, 1976; Madhavan and Schneiderman, 1977). The imaginal cells of the seven anterior segments are arranged in six clusters of histoblasts per segment which remain uninvaginated and do not undergo any detectable cell divisions during larval life. In contrast, the imaginal cells of the terminal segment are all combined into one large imaginal disc which is attached to the ventral midline and grows by cell division during most of larval development (Dübendorfer, K., 1977; Madhavan and Schneiderman, 1977). Comparative morphology and the study of intersexual flies have led to the hypothesis that the genital disc represents the fusion of the imaginal anlagen of several primitive insect segments, the female genitalia being formed by the imaginal cells of a primitive 8^{th} abdominal segment, the male genitalia by the imaginal cells of a 9^{th}, and the analia by the imaginal cells of a 10^{th} and/or possibly 11^{th} segment (Gleichauf, 1936; Dübendorfer, A., 1971). Although such a fusion may well have taken place sometime during the evolution of the species Drosophila mosaic flies provide no evidence that it is repeated during the embryonic development of the animal. The fate map of the female genital disc gives no indication of any right and left or dorsal and ventral clustering of the embryonic precursor cells. Already in the earliest stages the precursor cells of the genital disc are found in one small coherent region of the embryo mapping rather far apart from the precursors of the posteriormost histoblasts. The anterior-posterior order of female genitalia and analia is, however, reminiscent of the postulated primitive segmental organization. Gynandromorph data allow an estimation of the size of the total area from which in an embryo the precursor cells for the adult structure will be drawn. The frequency with which the female genital disc derivatives were mosaic in triplo-X-diplo-X mosaics (34.5%) led to a minimum

estimate of 15 cells for the total genital-anal precursor area at the blastoderm stage (for a discussion of cell number estimates see p. 29). Within such a precursor area there may, however, be cells that do not contribute to the adult structure studied, but instead give rise to larval structures or form unscoreable parts of the imaginal disc. Estimations based on the size of clones induced at the blastoderm stage (Schüpbach et al., in prep.) as well as the frequency with which mitotic recombination could be induced in the female genitalia and analia at the blastoderm stage (Dübendorfer, K., 1977) all point to a smaller number of blastoderm cells giving rise to the female genital disc derivatives. In female embryos some of the cells within the precursor area of the genital disc might therefore have a developmental fate different from forming adult female genital structures. The sturt distances between the female genitalia and analia are relatively large. This suggests that such cells - if they exist - must be situated in the central region of the total precursor area. In fact, the fate map derived from normal gynandromorphs indicated that the cells which occupy the area between female genitalia and analia can give rise to the male genitalia, if endowed with the proper genetic constitution (X/O). Taken as a whole these results are best interpreted in the way proposed by Nöthiger, et al., (1977): Within the embryonic precursor area of the genital disc three regions might be distinguished: the region most anterior would contain precursors for the female genital structures which in a male animal would not develop to any scoreable adult structures. The central region would contain precursors for the male genital structures which in a female would not give rise to scoreable adult structures, and the region most posterior (mapping closest to the germline as determined in the female mosaics; data not shown) would give rise to the analia of both male and female, depending on its genetic constitution.

In an earlier study of the mosaic patterns in the genital disc derivatives of normal gynandromorphs it has already been pointed out that the precursorsof male and female genitalia can sometimes be separated

by the mosaic border (Nöthiger et al., 1977). A separate position on the embryonic fate map does, however, not imply a cell lineage restriction between the different regions at the blastoderm stage. The sturt distances between female and male genitalia as well as the distances between male genitalia and analia which were measured in normal gynandromorphs were all small. Judging from these distances it might well be possible that clonal overlaps could occur between these precursor regions at the blastoderm stage. Indeed, the precursor regions for male and female genitalia have been shown to share common cells at the blastoderm stage as do the precursor regions for male penis structures and analia (Wieschaus and Nöthiger, in prep.). A cell lineage restriction is only observed between the male genital plate and analia and between female genitalia and analia (DÜbendorfer, K., 1977).

In contrast to the genital region only one group of cells seems to give rise to the analia. These cells would develop a female or a male pattern depending on their genotypic sex. In the normal gynandromorphs the anal plates always added up to one complete set. The total frequency of mosaicism in the anal plates was not different in female mosaics (16.7%) and in normal gynandromorphs (20.5%). However, in the female mosaics the right and left sides of the anal plates were more frequently mosaic and mapped closer to the ventral midline than in the normal gynandromorphs. This can be explained by assuming some cell mixing between the right and left halves of the female anal plates, which would not be found in the normal gynandromorphs. Normal growing blastoderm clones in fact overlapped right and left, as do $\underline{\text{Minute}}^{+}$-clones induced up to the end of the second larval instar (DÜbendorfer, K., 1977; Schüpbach et al., in prep.). In many of the triplo-X-diplo-X mosaic anal plates the mosaic border ran close to the line of symmetry producing overlap between the two sides only in a few bristles. The same has been observed in mosaic females which had genetically marked normal growing clones in their anal plates due to induced mitotic recombination (Ulrich, 1971;

Schüpbach *et al.*, in prep.). This suggests that cells may cross from one side to the other late in development after the right and left side of the anal plates have fused. This late overlap or mixing would not be expected to occur in normal gynandromorphs where cells do not fuse on the midline if they are of different genotype. Mixing or overlap would imply that until late in development right and left pattern can share common precursors and that a cell lineage restriction between the two sides must arise even later in development, if it exists at all.

3. The female germline

3.1. Number of precursor cells

The number of pole cells at the blastoderm stage observed in morphological studies ranges from 32 to 52 (Poulson, 1965; Turner and Mahowald, 1976; Zalokar and Erk, 1976). Based on clones induced in the germline, Wieschaus and Szabad (in press) have estimated the number of pole cells that become functional germ cells to be between 4 and 8 at the blastoderm stage. The data given in this study also allow an estimation of the number of presumptive germ cells at the blastoderm stage by comparing the frequency of mosaicism in the germline to that found in somatic structures, as was done in the case of the genital disc. Two structures which exhibit the same frequency of mosaicism should arise from equally large areas of embryonic cells or nuclei, as long as the nuclei divide at the same rate and in moving to the surface of the egg behave in the same way. For the pole cell nuclei as compared to the somatic nuclei this seems to be true only up to the 9^{th} division (Turner and Mahowald, 1976; Zalokar and Erk, 1976). In this study a single leg showed the same frequency of mosaicism as the total germ cell population of a female (Table 2). We may assume therefore that up to the 9^{th} division the leg precursor nuclei would be found in a similarly large area of nuclei as the precursors of the functional adult female germline. From then on the somatic nuclei divide faster than the pole cell nuclei. At the blastoderm stage the leg precursors therefore would now, after 3-4 more divisions, occupy a larger area of cells than the future stem cells which on average underwent only 1-2 more divisions. As mentioned before the precursor area of the single foreleg was estimated to cover about 10 cells at the blastoderm stage (Wieschaus and Gehring, 1976a, b) It is not easy to estimate the number of precursor nuclei at the 9^{th} division which gives rise to these 10 cells. Even one cell division before the blastoderm stage the 10 cells will not correspond to 5 precursor nuclei, but to some more, which may

give one or both their daughters to the group of 10 cells, since their cell lineage is not fixed. With small numbers of cells the simple division of this number by two yields in fact very inaccurate estimates of the number of precursors one cell division earlier. This becomes obvious, if one calculates the number of foreleg precursors in this manner back to the 9th division. At that stage the number of precursor nuclei would be estimated to be 0.675, whereas from the mosaic frequency of the foreleg it is known that even at the 1st division the foreleg had in about 75% of the cases 1 and in about 25% of the cases 2 precursor nuclei (= 1.25 nuclei on an average). A better estimate can be obtained if one outlines 10 adjacent cells on a continuous sheet of cells and makes random pairs of neighbouring cells over the total sheet which would represent the two daughters of one precursor cell. The 10 cells belong then on an average to 7 different pairs, corresponding to 7 precursor cells. Going back further one estimates that 7 cells (or nuclei) should on an average arise from 5 precursors. At the 9th division the 10 foreleg precursors might thus have 3-4 precursor cells, a number which should correspond to the number of cells or nuclei within the precursor area of the germline at that time. Since, in contrast to the somatic cells, in the case of the germline the cell lineage is from the 9th division on restricted such that in each cell division all daughter cells fall into the outlined group (the pole cells), one may in a very rough manner estimate that the precursor area of the female germline at the blastoderm stage contains 6-16 cells.

As has been pointed out (see legend to Table 7), an area of 10 cells as the precursor area of the single foreleg at the blastoderm stage may be a slight underestimate. This figure of 10 cells was derived from clonal analysis (Wieschaus and Gehring, 1976 a). X-rays are known to cause cell death, and in Drosophila it has been shown that the surviving cells can compensate for the loss by increased proliferation (Schweizer, 1972). This means that after X-irradiation a structure may

be formed from fewer cells than in unirradiated control animals. This decrease in the number of precursor cells is reflected in an increase in clone size. Haynie and Bryant (1977) have irradiated 72^{h} old larvae with increasing doses of X-rays. From their data, they concluded that a dose of 1000 r kills 40%-60% of the cells in the wing imaginal disc. It is certainly possible that a similar amount of cell death occurs in embryos irradiated at the blastoderm stage. Yet, whereas 90% of the larvae irradiated at 72^{h} emerged as adults (Haynie and Bryant, 1977), only 33.2% of the embryos irradiated at the blastoderm stage developed to adults (Wieschaus and Gehring, 1976 a). Among the large fraction of irradiated embryos which did not survive to adults one would expect to find those which suffered the highest amount of cell death. The cell death occurring in the surviving animals may therefore be less than 40%-60%. In fact, when embryos were irradiated at the blastoderm stage with 550 r and 1100 r respectively, no difference in clone size between the two series was observed in the derivatives of several imaginal discs. Therefore it appears that the higher dose at this stage did not cause a noticeable increase in cell death among the survivors (Wieschaus and Gehring, 1976 a, Table 3).

Another possibility for underestimating the number of cells in our calculation arises if in the precursor area of the foreleg cells are interspersed that do not contribute to adult structures. These cells would not be detected in a clonal analysis, but they would increase the frequency of mosaicism in gynandromorphs. Since the width (in the anterior-posterior direction of the embryo) of an entire thoracic segment has been estimated to be 3-4 cells at the blastoderm stage (Lohs-Schardin and Nüsslein, in prep.), and the precursor area of the foreleg should be more or less of circular shape, there seems to be little space for a large number of such undetectable cells within this precursor area (Wieschaus and Gehring, 1976 b).

Allowing, therefore, for some X-ray induced cell death and occasional

cells within the foreleg area which will not contribute to the adult structure, we could raise the estimate for the area of the foreleg to some 15-20 cells. Based on this, the numbers for the precursor area of the female germline would also slightly increase. We would arrive at 4-5 precursors at the 9^{th} division and thus to an area of 8-20 pole cells at the blastoderm stage among which the actual precursors of the female germline are distributed.

In some embryos the mosaic border will cut through the germline primordium such that only one of the initial founder cells is of the minority genotype. These cases will result in mature ovaries where the fraction of cysts of this minority genotype (i.e. the "patch size") will on the average represent the reciprocal of the total number of founder cells, in our case 1/6 to 1/16. By reversing the argument, it is sometimes possible to estimate the number of founder cells from the smallest patch sizes in mosaics (Stern, 1940). However, the fraction of cells of the minority genotype in our mosaic ovaries varied greatly, and we could find no indication of any clustering around one smallest size class, nor indeed around any other size class (Fig. 6). This is probably due to the variability in the proliferation of individual germ cells during development, coupled with the irregularity in the pattern of stem cell bursts. A choice of any particular patch size as representative of a single founder cell (Nissani, 1977b) would therefore be extremely arbitrary and we have refrained from making estimations of cell numbers based on patch size.

There are, of course, alternative ways of estimating numbers of cells using mosaic frequencies (Ripoll, 1972; Nissani and Lipow, 1977; Wyman and King, 1978; for reviews see Janning, 1978; and Merriam, 1978). In calculations where the egg is visualized as a sphere and the mosaic border as a great circle, a frequency of mosaicism of 25% requires a large number of blastoderm cells(54-108). These numbers are certainly overestimates. The mosaic border observed on the hypoderm

of larval gynandromorphs should be fairly representative of the situation in mosaic blastoderm embryos. This border is very convoluted and deviates strongly from a straight line (Szabad et al., in prep.). A great circle on a spherical egg therefore yields a gross underestimate of the length of the mosaic border. Janning (1978) has derived an alternative method for estimating cell numbers at the blastoderm stage which takes into consideration both the actual shape of the egg and the convolution of the border. It is based on a comparison of sturt distances measured along and across the embryo to the actual number of blastoderm cells observed in morphological studies (Turner and Mahowald, 1976; Zalokar and Erk, 1976). The length of the embryo was calculated to be 160 sturts, the width 84 sturts, which led to a diameter of a single blastoderm cell of 2 sturts. This is considerably smaller than our estimate of a blastoderm cell which is 5.7 sturts (Table 7). Even raising the number of blastoderm cells within the precursor area of the foreleg to 15 cells would still yield a diameter of 4.7 sturts per cell. The value of 160 sturts for the length of the embryo, or 84 sturts for its width seem, however, relatively small. It has been shown (Janning, 1978) that distances between two landmarks larger than 15 sturts are underestimates due to the convolution of the gynandromorph border. This is clearly seen in the fact that distances of 20 sturts and more, in particular, become much larger if they are derived by adding small distances rather than by measuring them directly (Janning, 1978). When the length of the embryo is determined in sturts, several such large distances have to be used, e.g. between the anterior tip of the embryo and the landmarks of the head, between the landmarks of the head and those of the thorax, between the 7^{th} abdominal tergite or sternite and posteriorly derived internal structures. These distances might in reality be considerably larger than what has been measured in gynandromorph analyses, and thus the length and similarly the width of the embryo in sturts could be considerably increased. In this way, the discrepancy between our estimates and those of Janning (1978) would become much smaller.

In conclusion, the cell numbers of 15 for the precursor area of the female genital disc and 6-16 for the precursor area of the germline may be minimum estimates. In any case, we regard the intellectually very stimulating problem of finding an apropriate method for translating mosaic frequencies into estimates of cell numbers as more intriguing than the question whether we are finally dealing with 10, 15 or even 20 precursor cells for a particular area at the blastoderm stage.

3.2. Cell lineage relationships

Within the group of germ cells no preferential genotypic borders were detected. The functional germ cells do not appear to be separated into two clusters, one for populating the right and one for the left gonad. There was no case of perfect right/left separation, and the 20 out of 379 cases where only one gonad was mosaic would amount to a distance of 3 sturts (10/379) between the centers of left and right gonad in the early embryo.

After having reached the embryonic gonad the germ cells divide logarithmically during larval development (Wieschaus and Szabad, in press). During the pupal stage they are integrated into the ovarioles which will form the adult ovary (King, 1970). In almost all ovaries which were mosaic we observed at least one ovariole which contained cysts of both genotypes. The stem cells in these germaria therefore possessed a pluralistic cell lineage back to the earliest nuclear divisions in the egg. This corresponds well with the results of Wieschaus and Szabad (in press) who also found mosaicism within single ovarioles after inducing mitotic recombination at the blastoderm stage.

3.3. Stem cell divisions in the adult

The stem cells in the germarium represent one of the few cell types in an adult Drosophila which continue to divide throughout the life of the animal. The pattern of cysts in mosaic ovarioles will reflect the sequence of these divisions as long as in the germarium no events such as mixing occur which would alter the arrangement of the cysts prior to their entry into the vitellarium. This assumption is supported by electron microscopic studies (Carpenter, 1975) which show that in the germarium the apical-basal order of the cysts does in fact correspond to their developmental age. In addition, the non-random clustering we have observed in our analysis cannot be reasonably explained by mixing, since this would actually lead towards randomness. The stem cell sequence could also be obscured if following the stem cell division additional divisions occurred to produce clusters of 2 or more cysts. There is no microscopic evidence for such an event. Moreover, Wieschaus and Szabad (in press) generated cysts homozygous for a genetic marker by X-ray induced mitotic recombination in the adult. They found that 50% of the clonal inductions resulted in single marked eggs and 50% in series of marked eggs. This bimodality is precisely that expected of asymmetric stem cell divisions and argues against the occurrence of subsequent additional divisions following the stem cell division. We therefore think that our results relate directly to the cell cycle of the stem cells themselves.

Ovarioles which contain only one stem cell in their germarium can, of course, never be mosaic, and if such ovarioles exist, they were therefore not included in the analysis. Ovarioles which had stem cells of different genotypes in their germarium, but by chance showed only cysts of one genotype in their vitellarium, were also not included in the analysis. A rough estimation of their number can be made by calculating the probability of obtaining no change in cyst genotype within five divisions using the observed frequency of neighbouring cysts with identical genotype. Thus, about 17% ($(0.7)^5$) of all ovarioles

with mosaicism in their stem cell population were classified as non-mosaic ovarioles. When these ovarioles are also taken into account , the frequencies of neighbouring cysts with alike genotype increases, and not only division series of two, but also such of three cysts become significantly more frequent (65%) than randomly expected. Series of four (56%) and even series of five (50%) cysts should occasionally occur.

Electron microscopic studies of single germaria describe 2 stem cells (King, 1970; Carpenter, 1975). Wieschaus and Szabad (in press) estimate from the frequency of mitotic recombination and clone size 2.8 stem cells per ovariole. Three stem cells is in fact more compatible with the patterns we obtained from our mosaic ovarioles, especially after having corrected for ovarioles with undetected mosaicism. With three stem cells, one hundred eggs would be made from 37 separate stem cell series. 14 of these would be "single" divisions leaving 23 bursts to account for the remaining 86 eggs. This yields an average burst size of 3.7. Similar calculations assuming two stem cells result in a burst size of 5.2 (18 bursts must account for 93 eggs).

The fact that the stem cells divide in bursts means that the length of the cell cycle from one mitosis to the next is very variable. Since both single cysts and series of cysts occur side by side in the same mosaic ovary, the factors controlling the patterns of stem cell division are probably neither environmental nor humoral. We are thus forced to conclude that the observed pattern derives from intrinsic properties of the stem cell population itself.

VI REFERENCES

Ananiev, E.V., Faizullin, L.Z., Gvozdev, V.A.: The role of genetic balance in control of transcription rate in the X-chromosome of Drosophila melanogaster. Chromosoma 45, 193-201 (1974)

Baker, P.S.: Paternal loss (pal): a meiotic mutant in Drosophila melanogaster causing loss of paternal chromosomes. Genetics 80, 267-296 (1975)

Beadle, G.W., Ephrussi, B.: Ovary transplants in Drosophila melanogaster: meiosis and crossing-over in superfemales. Proc. Nat. Acad. Sci., USA 23, 356-360 (1937)

Brehme, K.S.: Effects of the triplo-X condition on development in Drosophila melanoaster. Proc. Soc. Exp. Biol. and Med. 37, 578-580 (1937)

Bryant, P.J., Zornetzer, M.: Mosaic analysis of lethal mutations in Drosophila. Genetics 75, 623-637 (1973)

Carpenter, A.T.C.: Electron microscopy of meiosis in Drosophila melanogaster females. I. Structure, arrangement, and temporal change of the synaptonemal complex in wild-type. Chromosoma (Berlin) 51, 157-182 (1975)

Dobzhansky, Th.: Studies on the intersexes and supersexes in Drosophila melanogaster. Bull. Bur. genet. 8, 91-158 (1930)

Dübendorfer, A.: Untersuchungen zum Anlageplan und Determinationszustand der weiblichen Genital- und Analprimordien von Musca domestica. Wilhelm Roux'Arch. 168, 142-168 (1971)

Dübendorfer, K.: Die Entwicklung der männlichen und weiblichen Genital-Imaginalscheibe von Drosophila melanogaster: Eine klonale Analyse. Ph.D. Thesis, University of Zurich, Switzerland (1977)

Faizullin, L.Z., Gvozdev, V.A.: Dosage compensation of sex-linked genes in Drosophila melanogaster. Molec. Gen. Genet. 126, 233-245 (1973)

Fielding, C.J.: Developmental genetics of the mutant grandchildness of Drosophila subobscura. J. Embryol. Exp. Morph. 17, 375-384 (1967)

Fritz-Niggli, H.: Vergleichende Analyse der Strahlenschädigung von Drosophila-Eiern mit 180 keV und 31 MeV. Fortschr. Röntgenstr. 83, 178-200 (1955)

Garcia-Bellido, A., Merriam, J.: Cell lineage of the imaginal discs in Drosophila gynandromorphs. J. Exp. Zool. 170, 61-76 (1969)

Gehring, W., Wieschaus, E., Holliger, M.: The use of "normal" and "transformed" gynandromorphs in mapping the primordial germ cells and the gonadal mesoderm in Drosophila. J. Embryol. Exp. Morph. 35, 607-616 (1976)

Geigy, R.: Erzeugung rein imaginaler Defekte durch ultraviolette Eibestrahlung bei Drosophila melanogaster. Wilhelm Roux'Arch. Entwickl.-Mech. Org. 125, 406-447 (1931)

Gleichauf, R.: Anatomie und Variabilität des Geschlechtsapparates von Drosophila melanogaster Meig. Z.Zool. 148, 1-66 (1936)

Hadorn, E., Gloor, H.: Transplantationen zur Bestimmung des Anlagemusters in der weiblichen Genital-Imaginalscheibe von Drosophila. Rev. Suisse Zool. 53, 495-501 (1946)

Hall, J., Gelbart, W., Kankel, D.: Mosaic Systems. In: The Genetics and Biology of Drosophila (M. Ashburner and E. Novitski, eds.), Vol. I, pp. 264-314. London, New York: Academic Press (1976)

Haynie, J.L., Bryant, P.J.: The effects of X-rays on the proliferation dynamics of cells in the imaginal wing disc of Drosophila melanogaster. Wilhelm Roux's Archives 183, 85-100 (1977)

Hinton, C.W.: The behavior of an unstable ring-chromosome of Drosophila melanogaster. Genetics 40, 951-961 (1955)

Hotta, Y., Benzer, S.: Mapping of behavior in Drosophila mosaics. Nature 240, 527-535 (1972)

Hotta, Y., Benzer, S.: Mapping of behavior in Drosophila mosaics: In: Genetic Mechanisms of Development (F. Ruddle, ed.). New York: Academic Press (1973)

Huettner, A.F.: The origin of the germ cells in Drosophila melanogaster. J. Morphol. 37, 385-423 (1923)

Illmensee, K.: The potentialities of transplanted early gastrula nuclei of Drosophila melanoaster. Production of their imago descendants by germline transplantation. Wilhelm Roux'Archiv 171, 331-343 (1973)

Illmensee, K., Mahowald, A.P., Loomis, M.R.: The ontogeny of germ plasm during oogenesis in Drosophila. Develop. Biol. 49, 40-65 (1976)

Janning, W.: Aldehydeoxidase as a cell marker for internal organs in Drosophila melanogaster. Naturwiss. 59, 516-617 (1972)

Janning, W.: Entwicklungsgenetische Untersuchungen an Gynandern von Drosophila melanogaster. II. Der morphogenetische Anlageplan. Wilhelm Roux'Archiv 174, 349-359 (1974)

Janning, W.: Entwicklungsgenetische Untersuchungen an Gynandern von Drosophila melanogaster. IV. Vergleich der morphogenetischen Anlagepläne larvaler und imaginaler Strukturen. Wilhelm Roux's Archives 179, 349-372 (1976)

Janning, W.: Gynandromorph fate maps in Drosophila. In: Results and Problems in Cell Differentiation: Genetic Mosaics and Cell Differentiation (W. Gehring, ed.) Berlin-Heidelberg-New York: Springer Verlag (1978)

King, R.C.: Ovarian development in Drosophila melanogaster. New York: Academic press (1970)

Laugé, G.: Recherches expérimentales sur la détermination et la différenciation des caractères morphologiques et histologiques des intersexués triploides de Drosophila melanogaster Meig. Thèses, Faculté des Sciences, Université de Paris, France (1968)

Lindsley, D.L., Grell, E.H.: Genetic variations of Drosophila melanogaster. Carnegie Inst. Washington Publ. 627 (1968)

Lohs-Schardin, M., Nüsslein-Volhard, Ch.: A fate map or the larval hypoderm of Drosophila melanogaster: localized defects following irradiation of the blastoderm with a UV-laser microbeam.(in prep.)

Lucchesi, J.C.: Dosage compensation: Transcription-level regulation of X-linked genes in Drosophila. Am. Zool. 17, 685-694 (1977)

Lucchesi, J.C., Rawls, J.M. Jr., Maroni, G.: Gene dosage compensation in metafemales (3X;2A) of Drosophila. Nature 248, 564-567 (1974)

Madhavan, M.M., Schneiderman, H.A.: Histological analysis of the dynamics of growth of imaginal discs and histoblast nests

during the larval development of Drosophila melanogaster. Wilhelm Roux's Archives 183, 269-305 (1977)

Mahowald, A.P.: Ultrastructural observation on oogenesis in Drosophila. J. Morph. 137, 29-48 (1972)

Marsh, J.L., Wieschaus, E.: Germ-line dependence of the maroon-like maternal effect in Drosophila. Develop. Biol 60, 396-403 (1977)

Merriam, J.: Estimating primordial cell numbers in Drosophila imaginal discs and histoblasts. In: Results and Problems in Cell Differentiation: Genetic Mosaics and Cell Differentiation (W. Gehring, ed.) Berlin-Heidelberg-New York: Springer Verlag (1978)

Muller, H.J., League, B.B., Offerman, C.A.: Effects of dosage changes of sex-linked genes and the compensatory effect of other gene differences beween male and female. Anat. Rec. 51 (supp.) 110 (1931)

Nissani, M.: Cell lineage analysis of germ cells of Drosophila melanogaster. Nature 265, 729-731 (1977a)

Nissani, M.: A study of germinal mosaicism in Drosophila melanogaster. Wilhelm Roux's Archives 182, 203-211 (1977b)

Nissani, M., Lipow, C.: A method for estimating the number of blastoderm cells which give rise to Drosophila imaginal discs. Theor. Appl. Genet. 49, 3-8 (1977)

Nöthiger, R., Dübendorfer, A., Epper, F.: Gynandromorphs reveal two separate primordia for male and female genitalia in Drosophila melanogaster. Wilhelm Roux's Archives 181, 367-373 (1977)

Poulson, D.F.: The pole cells of Diptera, their fate and significance. Proc. Nat. Acad. Sci., USA 6, 182-184 (1947)

Poulson, D.F.: Histogenesis, organogenesis and differentiation in the embryo of Drosophila melanogaster, Meigen. In: Biology of Drosophila (M. Demerec, ed.), pp. 168-274, New York: Wiley, N.Y. 1965

Poulson, D.F.: Waterhouse, D.F.: Experimental studies on pole cells and midgut differentiation in Diptera. Australian J. Biol. Sci. 13, 541-567 (1960)

Ripoll, P.: The embryonic organization of the imaginal wing disk of Drosophila melanogaster. Wilhelm Roux's Archives 169, 200-215 (1972)

Schüpbach, R., Wieschaus, E., Nöthiger, R.: The embryonic organization of the genital disc studied in mosaics of Drosophila melanogaster.(in prep.)

Schweizer, P.: Wirkung von Röntgenstrahlen auf die Entwicklung der männlichen Genitalprimordien von Drosophila melanogaster und Untersuchung von Erholungsvorgängen durch Zellklon-Analyse. Biophysik 8, 158-188 (1972)

Seecof, R.L., Kaplan, W.D., Futch, D.G.: Dosage compensation of enzyme activities in Drosophila melanogaster. Proc. Nat. Acad. Sci. USA 62, 528-535 (1969)

Sonnenblick, B.P.: Germ cell movements and sex differentiation of the gonads in the Drosophila embryo. Proc. Nat. Acad. Sci., USA 27, 484-489 (1941)

Sonnenblick, B.P.: The early embryology of Drosophila melanogaster. In: Biology of Drosophila (M. Demerec, ed.), pp. 62-167, New York: Wiley (1950)

Stern, C.: The prospective significance of imaginal discs in Drosophila. J. Morph. 67, 107-122 (1940)

Stewart, B.R., Merriam, J.R.: Regulation of gene activity by dosage compensation at the chromosomal level in Drosophila. Genetics 79, 635-647 (1975)

Turner, R.F., Mahowald, A.P.: Scanning electron microscopy of Drosophila embryogenesis. 1. The structure of the egg envelopes and the formation of the cellular blastoderm. Develop. Biol. 50, 95-108 (1976)

Ulrich, E.: Cell lineage, Determination und Regulation in der weiblichen Genitalimaginalscheibe von Drosophila melanogaster. Wilhelm Roux' Arch. 167, 64-82 (1971)

Ursprung, H.: Untersuchungen zum Anlagemuster der weiblichen Genitalscheibe von Drosophila melanogaster durch UV-Strahlenstich. Rev. Suisse Zool. 64, 303-316 (1957)

Ursprung, H.: Fragmentierungs- und Bestrahlungsversuche zur Bestimmung von Determinationszustand und Anlageplan der Genitalscheiben von Drosophila melanogaster. Wilhelm Roux'Arch. Entwickl.-Mech. Org. 151, 504-558 (1959)

Wieschaus, E.: Clonal analysis of early development in Drosophila melanogaster. Thesis, Yale University, USA (1974)

Wieschaus, E.: Cell lineage relationships in the early Drosophila embryo. In: Results and Problems in Cell Differentiation: Genetic Mosaics and Cell Differentiation (W. Gehring, ed.) Berlin-Heidelberg-New York: Springer Verlag (1978)

Wieschaus, E., Gehring, W.: Clonal analysis of primordial disc cells in the early embryo of Drosophila melanogaster. Develop. Biol. 50, 249-263 (1976 a)

Wieschaus, E., Gehring, W.: Gynandromorph analysis of the thoracic disc primordia in Drosophila melanogaster. Wilhelm Roux's Archives 180, 31-46 (1976 b)

Wieschaus, E., Szabad, J.: Development and function of the female germline: a cell lineage study. Develop. Biol. (in press)

Wyman, R.J., King, D.G.: On the dispersion of imaginal progenitor cells in the Drosophila blastoderm. Genet. Res. Camb. 31, 273-286 (1978)

Zalokar, M., Erk, I.: Division and migration of nuclei during early embryogenesis of Drosophila melanogaster. J. Microsc. Biol. Cell. 25, 97-106 (1976)

TABLES and FIGURES

Table 1 Frequency with which certain adult structures or landmarks were of triplo-X genotype in 260 triplo-X-diplo-X mosaic females

adult structure	frequency (%) with which structures* were completely of triplo-X genotype	frequency (%) with which landmarks** (bristles, etc.) were of triplo-X genotype
eye	15%	27 %
antenna	12%	15 %
foreleg	2%	14 %
2nd leg	3%	17 %
3rd leg	6%	19 %
dorsal mesothorax	8%	17 % (wing) 23 % (scutellum)
humerus	18%	20 %
tergite 1	19%	32 %
tergites 2-5	33%	43 %
sternites 2-5	36%	41 %
derivatives of genital disc	18%	35 %
germ cells	54%	65 %***

* All these frequencies correspond to the adult structure on one side of the fly.

** For each adult structure several landmarks were scored, and the figures presented here are the average frequencies of these sets of landmarks.

*** Since in the germline of the ovary no constant landmarks can be defined this number was calculated adding 54% (ovaries in which all germ cells were triplo-X) + 0.46 · 22% (frequency of triplo-X cysts in mosaic ovaries).

Table 2 Frequency of mosaicism of certain adult structures (%) determined in a) 309 triplo-X-diplo-X mosaic females b) in 54 pal-induced $\hat{XX}/y^{+}Y.mal^{+}$- $\hat{XX}/O$ mosaic females and c) in 512 normal gynandromorphs.

adult structure	a triplo-X-diplo-X mosaic females		b pal-induced mosaic females		c normal gynandromorphs
foreleg	28.4		28.0		25.6
2nd leg	28.7		26.0		24.6
3rd leg	25.4		23.4		21.6
dorsal half-mesothorax	26.0		22.6		20.3
hemitergite 1	25.6		24.7		24.7
hemitergites 2-6	18.8		14.1		15.0
hemitergite 7	14.9		11.1		8.2
hemisternites 2-6	10.3		7.9		7.5
hemisternite 7	5.7		-		3.5
derivatives of the genital disc:					
total genital disc	34.5	28.2*	37.0	31.5*	26.9*
total genitalia	27.8	15.8*	33.3	22.5*	24.8*
total analia (including parovaria)	21.2	16.7*	28.8	18.5*	20.5*
genitalia on one side	15.2	6.6*	23.1	6.5*	18.0*
analia on one side (including parovaria)	13.1	9.6*	13.0	8.3*	4.7*
germ cell population:					
judged from cysts in ovaries	(n** = 354) 22.4		28.7		
judged from offspring	(n** = 129) 10.0		(n** = 43) 16.3		

* Only external structures were scored

** n= number of adults scored in these cases where n differed from the numbers given above.

Table 3 Egg production of triplo-X-diplo-X mosaic females and pal-induced $\hat{XX}/y^{+}Y.mal^{+}$- $\hat{XX}/0$ mosaic females with various composition of genitalia (irrespective of the genotypic constitution of the ovaries).

Genotype of genitalia	Fraction of egg-laying females	Average number of eggs laid per female in 10 days
Triplo-X	0.0 ($\frac{0}{37}$)	0.0
Diplo-X	0.98 ($\frac{115}{117}$)	142.7 ± 24.1
Mosaic	0.83 ($\frac{40}{48}$)	67.6 ± 55.1
$\hat{XX}/y^{+}Y.mal^{+}$	1.0 ($\frac{20}{20}$)	166.0 ± 76.4
$\hat{XX}/0$	1.0 ($\frac{11}{11}$)	142.7 ± 67.2
Mosaic	1.0 ($\frac{12}{12}$)	152.2 ± 69.7
Non-mosaic control ($\hat{XX}/Y$)	1.0 ($\frac{20}{20}$)	152.8 ± 62.3

Table 4 Egg production and progeny of triplo-X-diplo-X mosaic females with entirely diplo-X genitalia, of pal-induced $\hat{XX}/y^+Y.mal^+$ - $\hat{XX}/0$ mosaic females, and of regular $\hat{XX}$/Y-females (non-mosaic control).

a Genotype of germcells in ovary	b Fraction of egg - laying females	c Average number of eggs per fertile female in 10 days	d Fraction of eggs which reached cellular blastoderm	e Fraction of eggs from which a larva hatched	f Average number of adult offspring per fertile female in 10 days	
					absolute	as fraction of eggs
Triplo-X	$\frac{39}{40} = 0.98$	119.8 ± 66.0	0.41	0.07	2.9 ± 3.1	0.02
Diplo-X	$\frac{47}{47} = 1$	163.3 ± 88.3	0.63	0.58	38.5 ± 42.1	0.24
Mosaic	$\frac{18}{18} = 1$	137.7 ± 70.4	0.68	0.60	29.5 ± 26.5	0.21
$\hat{XX}/y^+Y.mal^+$	$\frac{21}{21} = 1$	178.5 ± 77.3	0.60	0.52	21.7 ± 22.0	0.12
$\hat{XX}/0$	$\frac{10}{10} = 1$	146.4 ± 69.1	0.68	0.64	38.4 ± 23.8	0.26
Mosaic	$\frac{15}{15} = 1$	152.7 ± 68.0	0.66	0.60	31.4 ± 20.2	0.20
Non-mosaic control ($\hat{XX}$,y v f mal/Y)	$\frac{20}{20} = 1$	152.8 ± 62.3	0.68	0.62	40.4 ± 38.0	0.26

Table 5 Sturt distances measured between pairs of landmarks within the derivatives of the female genital disc in 309 triplo-X-diplo-X mosaic females; (for abbreviations see Fig. 2). Extreme values are given in bold print.

	D_1	D_2	Sp	R	V_1	V_2	VP_1	VP_2	VP_3	P	Av_1	Av_2	Av_3	Ad_1	Ad_2	Ad_3	Ad_4
D_1	1.8*	0.1	4.2	3.5	6.6	6.5	6.9	7.2	**9.0**	12.3	13.2	13.4	13.8	15.1	14.4	14.4	**15.5**
D_2		1.8*	4.2	3.7	6.6	6.5	7.0	7.4	8.9	12.4	13.3	13.3	13.7	15.2	14.6	14.5	**15.5**
Sp			8.9*	5.6	7.4	7.4	7.8	7.3	7.7	12.7	13.1	12.8	12.3	11.7	13.0	13.3	13.6
R				3.5*	5.0	4.9	5.6	4.7	5.8	13.2	12.3	12.7	13.1	13.7	13.7	13.7	14.6
V_1					9.8*	0.7	3.1	3.0	3.7	9.8	11.9	11.6	11.9	11.6	12.2	11.4	12.4
V_2						10.5*	2.9	3.2	3.5	**9.4**	11.9	11.6	11.9	11.6	12.2	11.2	12.5
VP_1							10.2*	1.6	3.2	11.8	11.2	11.2	11.5	12.1	12.0	11.3	13.1
VP_2								12.6*	3.3	11.1	10.9	10.9	11.2	12.0	11.5	11.2	12.7
VP_3									6.6*	12.0	11.8	11.8	12.3	12.4	12.3	11.7	12.4
P										6.8*	5.8	5.0	4.5	6.6	6.7	6.3	6.5
Av_1											4.4*	1.2	2.7	5.0	4.4	5.2	4.1
Av_2												4.8*	1.5	5.6	5.1	5.9	4.4
Av_3													4.4*	5.5	4.2	5.8	4.1
Ad_1														9.8*	2.5	1.4	4.7
Ad_2															6.5*	2.4	2.8
Ad_3																8.9*	4.2
Ad_4																	6.2*

* : distances between the landmarks on the right and on the left side of the animal

Table 6 Sturt distances measured between pairs of landmarks within the derivatives of the female genital disc from 54 pal-induced XX/y^+Y.mal^+ - XX/O mosaic females; (for abbreviations see Fig. 2). Extreme values are given in bold print.

	D_1	D_2	Sp	R	V_1	V_2	VP_1	VP_2	VP_3	P	Av_1	Av_2	Av_3	Ad_1	Ad_2	Ad_3	Ad_4
D_1	3.6*	0.0	5.6	3.7	11.1	11.1	13.4	15.7	16.7	15.1	17.4	19.3	18.3	17.3	17.3	17.3	18.3
D_2		3.6*	5.6	3.7	11.1	11.1	13.4	15.7	16.7	15.1	17.4	19.3	18.3	17.3	17.3	17.3	18.3
Sp			13.0*	8.4	9.1	9.1	14.7	12.8	15.6	17.8	14.5	18.5	18.3	16.5	16.5	16.5	17.4
R				1.9*	10.0	10.0	11.7	12.5	12.8	18.1	16.5	18.5	17.4	18.5	18.5	18.5	19.3
V_1					18.4*	0.0	5.6	5.6	5.9	14.7	18.5	18.3	17.4	16.5	16.5	16.5	17.4
V_2						18.4*	5.6	5.6	5.9	14.7	18.5	18.3	17.4	16.5	16.5	16.5	17.4
VP_1							13.0*	4.6	4.6	13.5	15.4	17.4	15.8	16.7	16.7	16.7	17.6
VP_2								13.0*	4.6	15.4	15.4	17.4	15.8	16.7	16.7	16.7	17.6
VP_3									12.1*	17.4	18.3	17.6	16.7	17.6	17.6	17.6	18.3
P										10.8*	9.6	11.5	10.6	9.6	9.6	9.6	10.6
Av_1											7.3*	3.8	4.8	1.9	1.9	1.9	2.9
Av_2												9.2*	1.0	1.9	1.9	1.9	2.9
Av_3													5.5*	1.0	1.0	1.0	1.9
Ad_1														7.3*	0.0	0.0	1.0
Ad_2															7.3*	0.0	1.0
Ad_3																7.3*	1.0
Ad_4																	5.5*

* Distances between the landmarks on the right and on the left side of the animal.

Table 7 **Estimate*** of the number of precursor cells within the precursor area of genital disc derivatives at the blastoderm stage, based on the frequencies of mosaicism (f)

Derivatives	triplo-X-diplo-X mosaic females				normal gynandromorphs	
	internal and external structures		external structures		external structures	
	f in %	number of precursor cells	f in %	number of precursor cells	f in %	number of precursor cells
Total genital disc	34.5	14.7	28.2	9.8	26.9	11.0
Total genitalia	27.8	9.6	15.8	3.1	24.8	9.4
Total vaginal plates			15.8	3.1	17.4	4.6
Total analia	21.2	5.5	16.7	3.5	20.5	6.4
Genitalia on one side	15.2	2.9	6.6	0.5	18.0	4.9
Single vaginal plate			6.6	0.5	7.3	0.8
Analia on one side	13.1	2.1	9.6	1.1	4.7	0.3

* This estimate was obtained from the frequency of mosaicism as given in Table 2. Since the radius (r) in sturts of the total precursor area should approximate the frequency of mosaicism (f) divided by π (Hotta and Benzer, 1973) the precursor area of different strucutures can be expressed in sturts^2 ($\pi . r^2$). In triplo-X-diplo-X mosaics the foreleg was mosaic in 28.4% of the cases which would thus equal an area of 257 sturts^2. Based on independent other data (Wieschaus and Gehring, 1976a, b) this area contains around 10 adjacent blastoderm cells. A single blastoderm cell in a triplo-X-diplo-X mosaic would therefore measure 25.7 sturts^2. Dividing the different precursor areas by this area of one blastoderm cell one obtains an estimate of the total number of cells within these precursor areas of the genital disc derivatives. This is rather a minimum estimate, since 10 cells for the total precursor area of the foreleg is probably the lower limit (Wieschaus, 1978). In the case of the normal gynandromorphs the 10 blastoderm precursor cells for the foreleg correspond to a mosaic frequency of 25.6% which means an area of 20.9 sturts^2 for a single blastoderm cell and allows the same calculation.

Table 8 Sturt distances measured within the derivatives of the genital disc in 512 normal gynandromorphs (for abbreviations see Fig. 4).

a) Distances between the centers of the entire* precursor areas

	VP	Hyp	GP	P	AP
T8	1.7	3.7	4.0	4.6	6.2
VP		2.8	3.6	5.6	6.6
Hyp			1.3	2.8	3.8
GP				3.8	4.4
P					2.9

* total of right and left side

b) Distances between the centers of the precursor areas on one side of the animal

	T8	VP	Hyp	GP	P	AP
T8	15.3*	4.8	6.3	6.3	6.6	6.0
VP		10.1*	5.4	5.6	6.3	7.5
Hyp			10.1*	1.7	4.8	6.0
GP				10.3*	5.2	6.7
P					11.0*	5.3
AP						15.8*

* distance between the structures on the right and on the left side of the animal

Table 9 Number of different ovarioles found in mosaic ovaries of triplo-X-diplo-X mosaic females and pal-induced $\hat{XX}/y^+Y.mal^+$- $\hat{XX}/0$ mosaic females

Genotype of ovaries	Total number of females with mosaic ovaries	Total number of ovarioles* which contained:		
		a) only mal^+ cysts	b) only mal^- cysts	c) cysts of both genotypes
Triplo-X-Diplo-X ($\hat{XX}$/In(1)2, w^{vC}- $\hat{XX}$, y v f mal/0)	64	378 (26 %)	713 (48 %)	379 (26 %) with: 1050 mal^+ 1224 mal^- cysts
$\hat{XX}/y^+Y.mal^+$- $\hat{XX}$,y v f mal/0	15	142 (32 %)	146 (34 %)	146 (34 %) with: 447 mal^+ 429 mal^- cysts

* During preparation some of the ovarioles broke apart. Only intact ovarioles were analyzed and included in this table.

FIGURES

Fig. 1: Fate map of the female abdomen derived from triplo-X-diplo-X mosaic females. T: tergite, S: sternite, GD: genital disc, GL: germline. The radii (r) of the circles reflect the frequency (f) of mosaicism according to the formula $f = \pi . r$ (Hotta and Benzer, 1973).

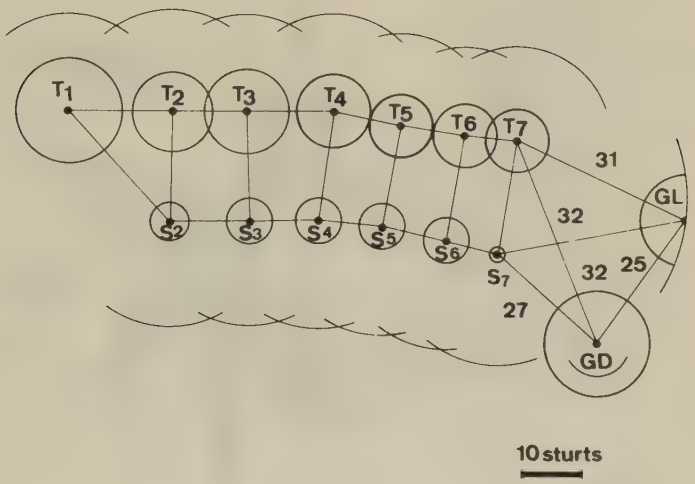
Fig. 2: The female genital disc. a) adult derivatives. D: oviduct, Sp: spermathecae, R: sperm receptacle, P: parovaria, V: vagina, VP: vaginal plates, T8: 8th tergite, Av: ventral analplate, Ad: dorsal anal plate. D_1, D_2 etc. : landmark bristles or points. b) fate map derived from triplo-X-diplo-X mosaic females (the landmarks correspond to those of Fig. 2a).

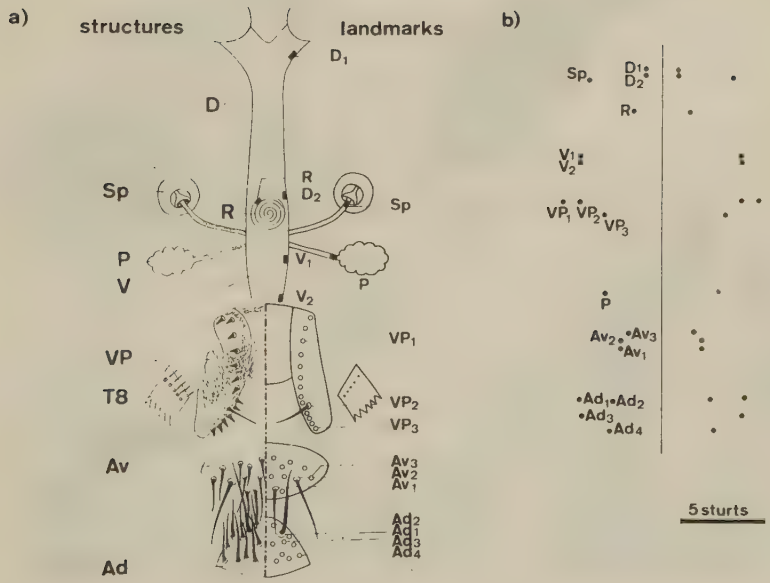
Fig. 3: The external derivatives of the male gential disc. GP: male genital plate, consisting of: GA, LP, C. GA: genital arch, LP: lateral plate, C: clasper, P: penis, Hyp: hypandrium, AP: anal plates (after Dübendorfer (1977).

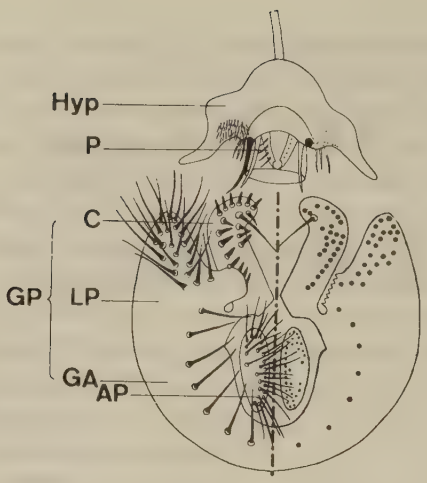
Fig. 4: Fate maps of the precursor areas of the genital disc derivatives. a) fate map of the external female structures, derived from triplo-X-diplo-X mosaic females. b) fate map derived from normal gynandromorphs. AP: anal plate, VP: vaginal plate, GP male genital plate, Hyp: hypandrium, P: penis, T8: 8th tergite. The radii (r) of the circles reflect the frequency of mosaicism (f) according to the formula $f = \pi r$.

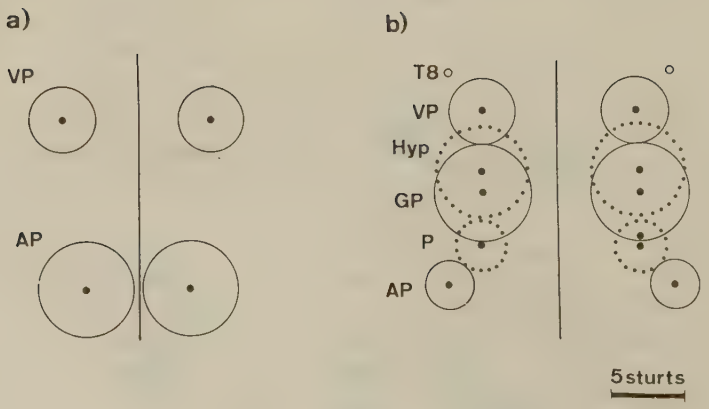
Fig. 5: Mosaic ovaries of triplo-X-diplo-X females which were histochemically stained for aldehydeoxidase (ALDOX) acitivity. Triplo-X oocyte-nurse cell cysts show ALDOX activity and are darkly stained, whereas diplo-X cysts lack ALDOX activity and remain unstained. a, b: mosaic ovaries consist in most cases of mosaic ovarioles as well as "pure" ovarioles which contain cysts of one genotype only. Ovary b was associated with triplo-X genitalia and accumulated a large number of mature eggs. c-f: single mosaic ovarioles often contain several oocyte-nurse cell cysts of the same genotype following each other and only rarely "single" cysts (arrow in 2c) G: germarium, O: oocyte-nurse cell cysts in the vitellaria of single ovarioles.

Fig. 6: Relative amount of diplo-X oocyte-nurse cell cysts in triplo-X-diplo-X mosaic ovaries. 64 females which exhibited mosaicism in their germline were classified according to the percentage of diplo-X cysts in their ovaries.


T1
T2
T3
T4
T5
T6
T7
S2
S3
S4
S5
S6
S7
31
32
32
25
27
GL
GD
10 sturts


a)
structures
landmarks
D1
D
Sp
R
R
D2
Sp
P
V1
V
P
V2
VP1
VP
T8
VP2
VP3
Av
Av3
Av2
Av1
Ad2
Ad1
Ad3
Ad4
Ad
b)
Sp
D1
D2
R
V1
V2
VP1
VP2
VP3
P
Av2
Av3
Av1
Ad1
Ad2
Ad3
Ad4
5 sturts


Hyp
P
C
GP
LP
GA
AP


a)
VP
AP
b)
T8
VP
Hyp
GP
P
AP
5sturts

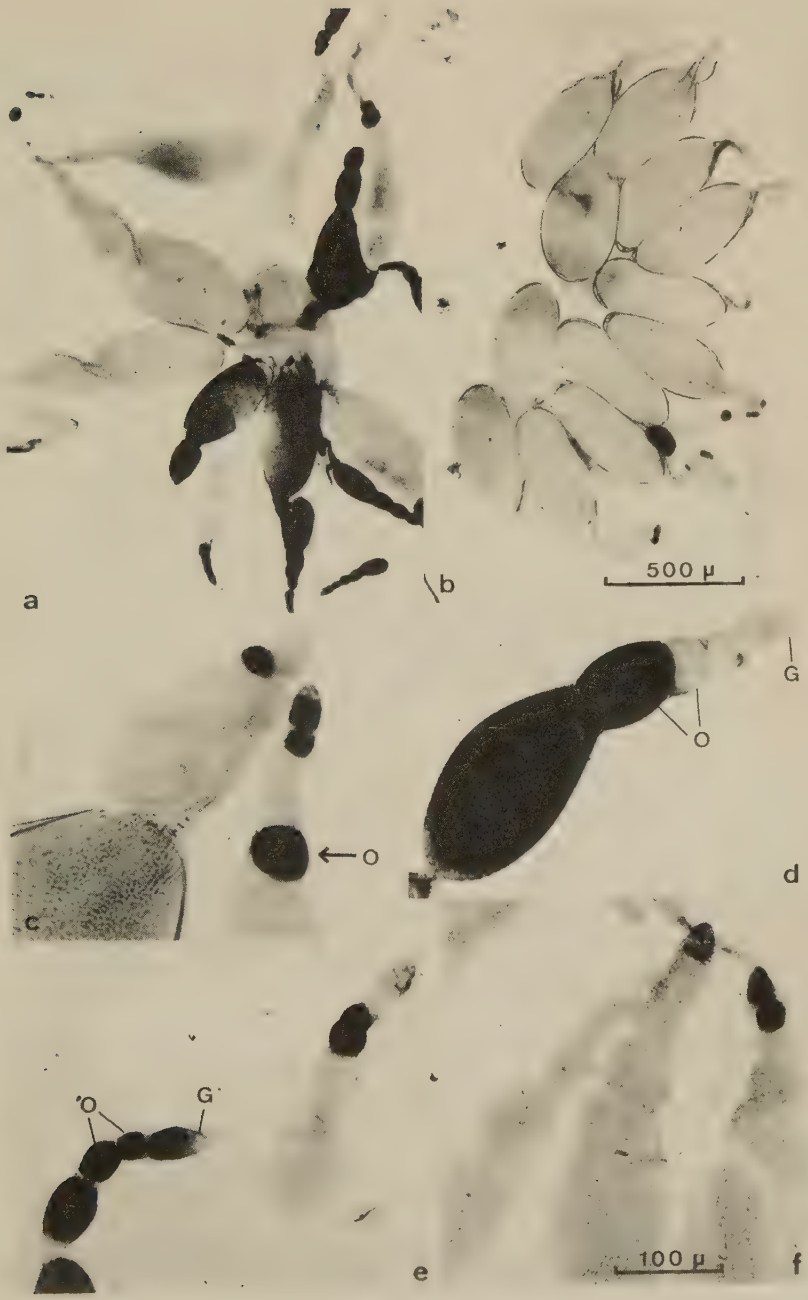
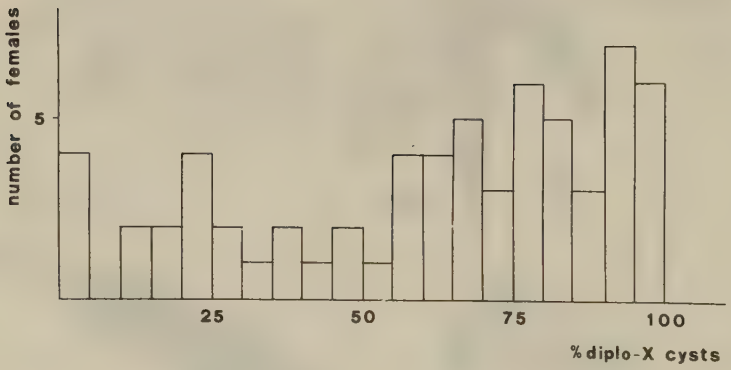


Fig. 5


number of females
5
25
50
75
100
% diplo-X cysts

CURRICULUM VITAE

Gertrud Maria Schüpbach-Seiler, von Grosshöchstetten (Kanton Bern), geboren am 3. Februar 1950. In Zürich besuchte ich die Primarschule, anschliessend trat ich ins Gymnasium der Töchterschule der Stadt Zürich ein, wo ich im Herbst 1969 das Maturitätszeugnis (Typus B) erwarb. Im Wintersemester 1969/70 begann ich das Biologiestudium an der Universität Zürich und bestand im Sommer 1974 die Diplomprüfung als Naturwissenschafterin mit Hauptfach Zoologie. Im Laufe meiner Studien besuchte ich Vorlesungen, Kurse und Seminarien der Dozenten K. Akert, R. Bachofen, Th. Baumann, J. Biegert, M. Birnstiel, H. Burla, P.S. Chen, C. Claude, C.D.K. Cook, I. Deak, A. Dübendorfer, M. Egli, C.H. Eugster, E. Hadorn, H. Hediger, H.-R. Hohl, R. Hunsperger, E. Jungen-Hauschteck, H. Jungen, S. Kubik, E. Kubli, H. Kummer, R. Nöthiger, P. Nüesch, H.-R. Oswald, D. Rast, O. Rohweder, J. Schlittler, H. Schmid, P. Tardent, H. Tobler, B.L. van der Waerden, H. Wanner, R. Wehner, C. Weissmann und V. Ziswiler. Seit April 1975 arbeite ich als Assistentin am Zoologischen Institut der Universität Zürich. Die vorliegende Dissertation entstand unter der Leitung von Prof. Dr. R. Nöthiger.